REMARKS/ARGUMENTS

The specification has been amended to remove the group X1, from the Scheme at the top of page 27, and from numbered paragraph [0074]. Support for this amendment is found, for example, at numbered paragraph [0076].

Claims 10-12 are new.

Support for each new and amended claim is found throughout the specification and at the originally filed claims. Additionally, support for amended Claims 8 and 10-11 is found at the Scheme at page 27. Support for Claim 12 is found at the Scheme at page 23.

Upon entry of the amendment, Claims 1-12 will be active.

No new matter is believed to have been added.

Applicants thank Examiner Aulakh for the helpful and courteous discussion of December 14, 2006, wherein amendments to the claims to address the indefinitness rejection, and wherein providing references to address the enablement rejection of Claim 9, were discussed.

Favorable reconsideration of the claims is respectfully requested in light of the amendments and arguments presented herein.

The indefiniteness rejection of Claims 1-9 is believed to be obviated by amendments to these claims. The term "derivative" has been removed from the claims, and in its place, the term "a benzylamine or its salt" has been inserted. The two offending parentheses of Claim 1, described at page 4 of the Official Action, have been deleted. Claims 6 and 7 are now drawn to a composition. Claim 8, a method claim, presents a synthetic Scheme and the appropriate steps to achieve the synthetic Scheme. Withdrawal of the rejection is respectfully requested.

The enablement rejection of Claim 9 is respectfully traversed. Applicants have submitted, along with the paper, abstracts and references which attest to the state of the art at

the time of filing of the application. The references describe that NK1/NK2 receptor antagonists are useful for treating the disease states described in Claim 9.

For example, Groneberg, at page 11, left column, lines 14-18, describe that the NK1/NK2 receptor antagonist DKN333 inhibited bronchioconstriction in asthma patients in a randomized, double-blind, placebo-controlled, cross over trial. Thus, NK1/NK2 receptor antagonists have use in (i.e., are enabled for) treating at least one obstructive bronchial disease.

Further, Okano (please see Okano's abstract) describe that "NK1 receptors play a role in mediating visceral pain, thus showing that the compounds are enabled for treating pain."

In the same paragraph, Okano describes that "TAK-637, an NK1 receptor antagonist, may be useful in treating functional bowel disorders such as Irritable Bowel Syndrome."

Polley (please the abstract of Polley) describes that "GR205171 is a potent antagonist of NK1 which mediated cranial vasodialation, dural PPE and expression of c-fos in the trigeminal nucleus caudalis," and as such, "has potential as a novel therapeutic agent in the treatment of migraine (e.g., a headache)."

Tattersall (please see the abstract of Tattersall) describes that "MK-0869 and its prodrug, L-758,298, have good activity against cisplatin-induced emesis (i.e., vomiting) in ferrets" and "provided a basis for the clinical testing of these drugs for treatment of emesis associated with cancer chemotherapy."

Further, Lecci (please see the abstract of Lecci) describe that "NK2 receptors are expressed in the gastrointestinal tract of both animals and humans, and that blockade (i.e., antagonism) of NK2 receptors should be considered as a viable mechanism for decreasing....[Irritable Bowel Syndrome]."

Finally, Vassout describes that NKP608, "a specific and potent antagonist at the neurokinin-1 (NK-1) receptor both in vitro and in vivo, ...exhibits an anxiolytic-like effect."

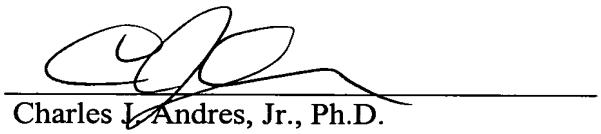
Please see the abstract of Vassout.

Accordingly, Applicants respectfully submit that because the compounds of the instant invention antagonize NK receptors, and because the state of the art at the time of the applications filing shows that NK receptor antagonists are useful for treating Irritable Bowel Syndrome, pain, anxiety, an obstructive bronchial disease (e.g., asthma), headache (e.g., migraine) and vomiting, Applicants respectfully submit that Claim 9 is enabled. Withdrawal of the rejection is respectfully requested.

Applicants submit the present application is now in condition for allowance. Early notification to this effect is earnestly solicited.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.
Norman F. Oblon



Charles J. Andres, Jr., Ph.D.
Attorney of Record
Registration No. 57,537

Customer Number
22850

Tel: (703) 413-3000
Fax: (703) 413 -2220
(OSMMN 06/04)

1: Regul Pept. 2000 Dec 22;96(1-2):7-16.

ELSEVIER FULL-TEXT ARTICLE Links

NKP608: a selective NK-1 receptor antagonist with anxiolytic-like effects in the social interaction and social exploration test in rats.

Vassout A, Veenstra S, Hauser K, Ofner S, Brugger F, Schilling W, Gentsch C.

Pharma Novartis AG, Nervous System, Research, WSJ386-2.45, CH-4002, Basel, Switzerland.

NKP608 is a non-peptidic derivative of 4-aminopiperidine which acts as a selective, specific and potent antagonist at the neurokinin-1 (NK-1) receptor both in vitro and in vivo. In vitro, the binding of NKP608 to bovine retina was characterized by an IC50 of 2.6+/-0.4 nM, whereas the compound's affinity to other receptor binding sites, including NK-2 and NK-3, was much lower. Species differences in IC (50) values with NKP608 were less pronounced than with previously described NK-1 receptor antagonists, being 13+/-2 and 27+/-2 nM in gerbil midbrain and rat striatum, respectively. In vivo, using the hind foot thumping model in gerbils, NKP608 exhibited a potent NK-1 antagonistic activity following oral administration (ID(50)=0.23 mg/kg; 2 h pretreatment), supporting a central activity of NKP608. The compound had a long duration of action with an ID(50) value of 0. 15 mg/kg p.o. and 0.38 mg/kg p.o. following a pretreatment of 5 and 24 h, respectively. Following a subchronic administration for 7 consecutive days (once daily) there was no evidence for the development of tolerance or accumulation. In the social interaction test performed in a highly illuminated, unfamiliar test arena, NKP608 specifically increased the time the two rats spent in social contact, and there was no concomitant increase in parameters reflecting general activity, i.e. ambulation (number of square entries) or the number of rearings. Active social time was maximally increased at a dose range of 0.01-1 mg/kg p.o. NKP608, the effect being weaker or absent at both lower (0.001 mg/kg p.o.) and higher (10 mg/kg p.o.) doses. A comparable bell-

shaped dose-response relation was seen in the social exploration test in rats. In this modified resident/intruder paradigm, maximal increase in social contact of the intruder rat directed towards the resident rat was seen at a similar dose range (0.03-3 mg/kg p.o.) The effects observed following an acute oral administration of NKP608 were comparable to those seen following a treatment with the well-known benzodiazepine, chlordiazepoxide, in both these tests. These findings indicate that NKP608 exhibits an anxiolytic-like effect and that this effect, as concluded from the observed antagonism of the hind foot thumping induced by i.c.v. administration of the NK-1 receptor agonist SPOMe, is centrally mediated. This makes this compound a potentially promising candidate for treating anxiety-related disorders in humans.

PMID: 11102646 [PubMed - indexed for MEDLINE]

Titre du document / Document title
The activity of GR205171, a potent non-peptide tachykinin NK[1] receptor antagonist, in the trigeminovascular system

Auteur(s) / Author(s)
POLLEY J. S. (1) ; GASKIN P. J. (1) ; PERREN M. J. (1) ; CONNOR H. E. (2) ; WARD P. (3) ; BEATTIE D. T. (1) ;

Affiliation(s) du ou des auteurs / Author(s) Affiliation(s)
(1) Systems Biology, Glaxo Wellcome Medicines Research Centre, Gunnels Woods Road, Stevenage, Herts SG1 2NY, ROYAUME-UNI
(2) Receptor Pharmacology, Glaxo Wellcome Medicines Research Centre, Gunnels Woods Road, Stevenage, Herts, SG1 2NY, ROYAUME-UNI
(3) Medicinal chemistry, Glaxo Wellcome Medicines Research Centre, Gunnels Wood Road, Stevenage, Herts SG1 2NY, ROYAUME-UNI

Résumé / Abstract
The in vivo activity of GR205171, a novel, highly potent non-peptide tachykinin NK[1] receptor antagonist, has been investigated in the trigeminovascular system in order to assess its potential as an acute therapy for migraine headache. In anaesthetised rabbits. CR205171 attenuated reductions in carotid arterial vascular resistance evoked by the tachykinin NK[1] receptor agonist. substance P methyl ester (SPOMe), injected via the lingual artery (DR[30] (i.e., the dose producing a dose-ratio of 30)=0.4 μg/kg, i.v.). In anaesthetised rats. CR205171 (0.1 and I mg/kg, i.v.) produced a dose-dependent inhibition of plasma protein extravasation (PPE) in dura mater. conjunctiva, eyelid and lip in response to electrical stimulation of the trigeminal ganglion. In anaesthetised guinea-pigs, GR205171 (1. 10 and 100 μg/ kg. i.v.) inhibited, by up to approximately 60%, expression of c-fos in the trigeminal nucleus caudalis in response to electrical stimulation of the trigeminal ganglion. It is concluded that GR205171 is a potent antagonist of NK[1] receptor-mediated cranial vasodilatation, dural PPE and expression of c-fos in the trigeminal nucleus caudalis. Such a profile of action suggests that GR205171 may have potential as a novel therapeutic agent in the treatment of migraine headache.

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Regulatory peptides (Regul. pept.) **ISSN** 0167-0115 **CODEN** REPPDY

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1997, vol. 68, n°1, pp. 23-29 (25 ref.)

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Mots-clés anglais / English Keywords
Tachykinin ; NK1 receptor ; Antagonist ; Trigeminal nucleus ; Treatment ; Chemotherapy ; Migraine ; Pain ; Animal ; Extravasation ; Brain (vertebrata) ; Central nervous system ; Nervous system diseases ; Central nervous system disease ; Cerebral disorder ; Cerebrovascular disease ; Cardiovascular disease ; Vascular disease ;

Mots-clés français / French Keywords
Tachykinine ; Récepteur tachykinine NK1 ; Antagoniste ; Noyau trijumeau ; Traitement ; Chimiothérapie ; Migraine ; Douleur ; Animal ; Extravasation ; GR205171 ; Encéphale ; Système nerveux central ; Système

nerveux pathologie ; Système nerveux central pathologie ; Encéphale pathologie ; Cérébrovasculaire pathologie ; Appareil circulatoire pathologie ; Vaisseau sanguin pathologie ;

002b02f04 ;

Mots-clés espagnols / Spanish Keywords
Taquikinina ; Receptor taquikinina NK1 ; Antagonista ; Núcleo trigeminal ; Tratamiento ; Quimioterapia ; Jaqueca ; Dolor ; Animal ; Extravasación ; Encéfalo ; Sistema nervioso central ; Sistema nervioso patología ; Sistema nervosio central patología ; Encéfalo patología ; Vaso sanguíneo encéfalo patología ; Aparato circulatorio patología ; Vaso sanguíneo patología ;

Localisation / Location
INIST-CNRS, Cote INIST : 18854, 35400006447552.0040

N° notice refdoc (ud4) : 2602626

Titre du document / Document title
Tachykinin NK2 receptor antagonists for the treatment of irritable bowel syndrome

Auteur(s) / Author(s)
LECCI Alessandro (1) ; CAPRIATI Angela (1) ; MAGGI Carlo Alberto (1) ;

Affiliation(s) du ou des auteurs / Author(s) Affiliation(s)
(1) Clinical Research Department, Menarini Ricerche via Sette Santi 1, 50131 Firenze, ITALIE

Résumé / Abstract
Tachykinin NK2 receptors are expressed in the gastrointestinal tract of both laboratory animals and humans. Experimental data indicate a role for these receptors in the regulation of intestinal motor functions (both excitatory and inhibitory), secretions, inflammation and visceral sensitivity. In particular, NK2 receptor stimulation inhibits intestinal motility by activating sympathetic extrinsic pathways or NANC intramural inhibitory components, whereas a modulatory effect on cholinergic nerves or a direct effect on smooth muscle account for the NK2 receptor-mediated increase in intestinal motility. Accordingly, selective NK2 receptor antagonists can reactivate inhibited motility or decrease inflammation- or stress-associated hypermotility. Intraluminal secretion of water is increased by NK2 receptor agonists via a direct effect on epithelial cells, and this mechanism is active in models of diarrhoea since selective antagonists reverse the increase in faecal water content in these models. Hyperalgesia in response to intraluminal volume signals is possibly mediated through the stimulation of NK2 receptors located on peripheral branches of primary afferent neurones. NK2 receptor antagonists reduce the hyper-responsiveness that occurs following intestinal inflammation or application of stressful stimuli to animals. Likewise, NK2 receptor antagonists reduce intestinal tissue damage induced by chemical irritation of the intestinal wall or lumen. In healthy volunteers, the selective NK2 antagonist nepadutant reduced the motility-stimulating effects and irritable bowel syndrome-like symptoms triggered by intravenous infusion of neurokinin A, and displayed other characteristics that could support its use in patients. It is concluded that blockade of peripheral tachykinin NK2 receptors should be considered as a viable mechanism for decreasing the painful symptoms and altered bowel habits of irritable bowel syndrome patients.

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British journal of pharmacology (Br. j. pharmacol.) **ISSN** 0007-1188 **CODEN** BJPCBM

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2004, vol. 141, n°8, pp. 1249-1263 [15 page(s) (article)] (3 p.1/4)

Langue / Language
Anglais

Editeur / Publisher
Nature Publishing, Basingstoke, ROYAUME-UNI (1968) (Revue)

Mots-clés anglais / English Keywords
Tachykinin receptor ; Tachykinin ; Neuropeptide ; Digestive diseases ; Intestinal disease ; Inflammation ; Sensitivity ; Secretion ; Nepadutant ; Substance P ; Irritable bowel syndrome ; Treatment ; Antagonist ; NK2 Tachykinin receptor ; Saredutant ;

Mots-clés français / French Keywords
Récepteur tachykinine ; Tachykinine ; Neuropeptide ; Appareil digestif pathologie ; Intestin pathologie ; Inflammation ; Sensibilité ; Sécrétion ; Népadutant ; Substance P ; Côlon irritable ; Traitement ; Antagoniste ; Récepteur tachykinine NK2 ; Sarédutant ;

002b02 ;

Mots-clés espagnols / Spanish Keywords
Receptor taquikinina ; Taquikinina ; Neuropéptido ; Aparato digestivo patología ; Intestino patología ; Inflamación ; Sensibilidad ; Secreción ; Nepadutant ; Substancia P ; Colón irritable ; Tratamiento ; Antagonista ; Receptor taquikinina NK2 ; Saredutant ;

Mots-clés d'auteur / Author Keywords
Neurokinin A ; substance P ; nepadutant ; saredutant ; irritable bowel syndrome ; intestinal motility ; intestinal secretion ; intestinal distension ; visceral sensitivity ; inflammation ;

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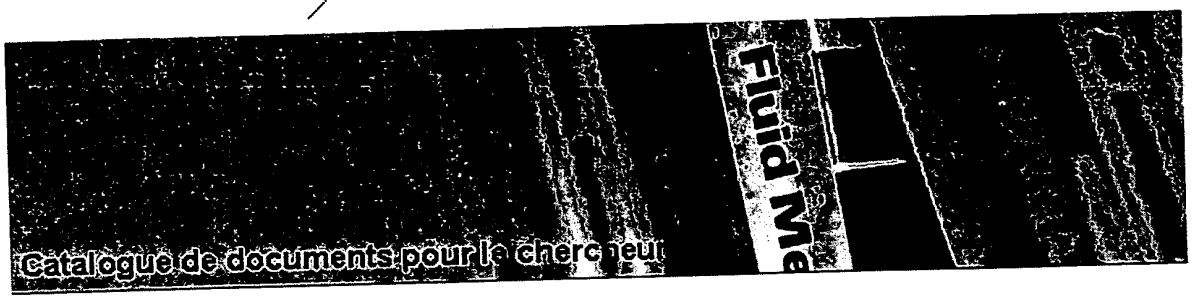
INIST-CNRS, Cote INIST : 4509, 35400011023950.0020

N° notice refdoc (ud4) : 15705589



Titre du document / Document title
Effects of tachykinin NK[1] receptor antagonists on the viscerosensory response caused by colorectal distention in rabbits

Auteur(s) / Author(s)
OKANO Shiho ; IKEURA Yoshinori ; INATOMI Nobuhiro ;

Résumé / Abstract
Irritable bowel syndrome (IBS) is a common disorder mainly characterized by altered bowel habits and visceral pain. In this study, we investigated the role of tachykinin NK[1] receptors in the visceral pain response (abdominal muscle contraction) caused by colorectal distention in rabbits previously subjected to colonic irritation, using the selective tachykinin NK[1] receptor antagonists TAK-637 [(aR,9R)-7-[3,5-Bis (trifluoromethyl)benzyl]-8,9,10,11-tetrahydro-9-methyl-5-(4 methylphenyl)-7H-[1,4] diazocino[2,1-g][1,7] naphthyridine-6,13-dione] and (′)-CP-99,994 (′)-(2S,3S) 3-(2-methoxybenzylamino)-2-phenylpiperidine. Intracolorectal administration of 0.8% acetic acid solution enhanced the nociceptive response to colorectal distention, producing a significant increase in the number of abdominal muscle contractions. Under these conditions, intraduodenal TAK-637 (0.1-3 mg/kg) dose dependently decreased the number of distention-induced abdominal contractions, and a significant inhibitory effect was observed with doses of 0.3 to 3 mg/kg. Another tachykinin NK[1] antagonist, (′)-CP-99,994, also reduced the number of abdominal contractions. In contrast, the enantiomer of TAK-637 (which has very weak tachykinin NK[1] receptor antagonistic activity), trimebutine maleate, ondansetron, and atropine sulfate did not inhibit the abdominal response. The main metabolite of TAK-637, which has more potent tachykinin NK[1] receptor antagonistic activity but permeates the central nervous system less well than TAK-637, produced less inhibition of the viscerosensory response. When given intrathecally, TAK-637 and (′)-CP-99,994 markedly reduced the number of abdominal contractions. These results suggest that tachykinin NK[1] receptors play an important role in mediating visceral pain and that TAK-637 inhibits the viscerosensory response to colorectal distention by antagonizing tachykinin NK[1] receptors, mainly in the spinal cord. They also suggest that TAK-637 may be useful in treating functional bowel disorders such as IBS.

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Titre du document / Document title
The novel NK[1] receptor antagonist MK-0869 (L-754,030) and its water soluble phosphoryl prodrug, L-758,298, inhibit acute and delayed cisplatin-induced emesis in ferrets

Auteur(s) / Author(s)
TATTERSALL F. D. [(1)] ; RYCROFT W. [(1)] ; CUMBERBATCH M. [(1)] ; MASON G. [(1)] ; TYE S. [(1)] ; WILLIAMSON D. J. [(1)] ; HALE J. J. [(2)] ; MILLS S. G. [(2)] ; FINKE P. E. [(2)] ; MACCOSS M. [(2)] ; SADOWSKI S. [(2)] ; BER E. [(2)] ; CASCIERI M. [(2)] ; HILL R. G. [(1)] ; MACINTYRE D. E. [(2)] ; HARGREAVES R. J. [(1)] ;

Affiliation(s) du ou des auteurs / Author(s) Affiliation(s)
[(1)] Department of Pharmacology, Merck, Sharp and Dohme, Neuroscience Research Centre, Terlings Park, Harlow, Essex, CM20 2QR, ROYAUME-UNI
[(2)] Merck Research Laboratories, Rahway, NJ, ETATS-UNIS

Résumé / Abstract
The anti-emetic profile of the novel brain penetrant tachykinin NK[1] receptor antagonist MK-0869 (L-754,030) 2-(R)-(1-(R)-(3,5-bis(trifluoromethyl)phenylethoxy)-3-(S)-(4-fluoro)phenyl-4-(3-oxo-1,2,4-triazol-5-yl) methylmorpholine and its water soluble prodrug, L-758,298, has been examined against emesis induced by cisplatin in ferrets. In a 4 h observation period, MK-0869 and L-758,298 (3 mg/kg i.v. or p.o.) inhibited the emetic response to cisplatin (10 mg/kg i.v.). The anti-emetic protection afforded by MK-0869 (0.1 mg/kg i.v.) was enhanced by combined treatment with either dexamethasone (20 mg/kg i.v.) or the 5-HT[3] receptor antagonist ondansetron (0.1 mg/kg i.v.). In a model of acute and delayed emesis, ferrets were dosed with cisplatin (5 mg/kg i.p.) and the retching and vomiting response recorded for 72 h. Pretreatment with MK-0869 (4-16 mg/kg p.o.) dose-dependently inhibited the emetic response to cisplatin. Once daily treatment with MK-0869 (2 and 4 mg/kg p.o.) completely prevented retching and vomiting in all ferrets tested. Further when daily dosing began at 24 h after cisplatin injection, when the acute phase of emesis had already become established, MK-0869 (4 mg/kg p.o. at 24 and 48 h after cisplatin) prevented retching and vomiting in three out of four ferrets. These data show that MK-0869 and its prodrug, L-758,298, have good activity against cisplatin-induced emesis in ferrets and provided a basis for the clinical testing of these agents for the treatment of emesis associated with cancer chemotherapy.

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Neuropharmacology (Neuropharmacology) **ISSN** 0028-3908 **CODEN** NEPHBW

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Mots-clés anglais / English Keywords
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Mots-clés français / French Keywords
Antagoniste ; Récepteur tachykinine NK1 ; Antiémétique ; Ondansétron ; Récepteur sérotoninergique 5-HT3 ; Dexaméthasone ; Hormone stéroïde ; Vomissement ; Association médicamenteuse ; Interaction

médicamenteuse ; Relation dose réponse ; Activité biologique ; Interaction moléculaire ; Prévention ; Animal ; Furet ; Voie intraveineuse ; Voie orale ; Corticostéroïde ; MK 0869 ; L 758298 ; Fissipedia ; Carnivora ; Mammalia ; Vertebrata ; Appareil digestif pathologie ;

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Mots-clés espagnols / Spanish Keywords
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Review article

Neurogenic mechanisms in bronchial inflammatory diseases

D. A. Groneberg[1], D. Quarcoo[1], N. Frossard[2], A. Fischer[1]

[1]Otto-Heubner-Centre, Pneumology and Immunology, Charité School of Medicine, Free University Berlin and Humboldt-University Berlin, Berlin, Germany; [2]EA 3771 «Inflammation and environment in asthma», Universite Louis Pasteur, Strasbourg I, Faculte de Pharmacie, France

Neurogenic inflammation encompasses the release of neuropeptides from airway nerves leading to inflammatory effects. This neurogenic inflammatory response of the airways can be initiated by exogenous irritants such as cigarette smoke or gases and is characterized by a bi-directional linkage between airway nerves and airway inflammation. The event of neurogenic inflammation may participate in the development and progression of chronic inflammatory airway diseases such as allergic asthma or chronic obstructive pulmonary disease (COPD). The molecular mechanisms underlying neurogenic inflammation are orchestrated by a large number of neuropeptides including tachykinins such as substance P and neurokinin A, or calcitonin gene-related peptide. Also, other biologically active peptides such as neuropeptide tyrosine, vasoactive intestinal polypeptide or endogenous opioids may modulate the inflammatory response and recently, novel tachykinins such as virokinin and hemokinins were identified. Whereas the different aspects of neurogenic inflammation have been studied in detail in laboratory animal models, only little is known about the role of airway neurogenic inflammation in human diseases. However, different functional properties of airway nerves may be used as targets for future therapeutic strategies and recent clinical data indicates that novel dual receptor antagonists may be relevant new drugs for bronchial asthma or COPD.

Key words: allergens; asthma; chronic obstructive pulmonary disease; nerves; tachykinins.

D. A. Groneberg
Pneumology and Immunology, Otto-Heubner-Centre
Augustenburger Platz 1 OR-1
D-13353 Berlin
Germany

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The release of neuropeptides such as the tachykinin substance P or calcitonin gene-related peptide (CGRP) can lead to inflammatory effects in the respiratory tract. The concepts of neurogenic inflammatory events in the airways and their potential importance for pulmonary diseases have been established since more than a decade (Fig. 1) (1). The initial activation of airway nerves relies upon interactions between receptors expressed on the terminal varicosities of airway nerve fibers with endogenous inflammatory mediators or with exogenous irritants such as tobacco smoke or other air pollutants. This activation may end after orthodromic central and antidromic local reflex pathways to signs of airway inflammation, bronchoconstriction and airway remodeling (2). Next to the large conducting airways also the small airways which have been reported to be important in airway inflammation (3) are abundantly innervated with a large number of neuromediators being expressed (4).

Because of the multitude of effects exerted by peptidergic neuromediators, a bi-directional linkage between cellular and molecular events of airway inflammation and the airway innervation exists (4, 5). These interactions are orchestrated by a large number of neuromediators such as tachykinins (6), CGRP, vasoactive intestinal polypeptide (VIP), gaseous molecules such as nitric oxide or carbon monoxide (7, 8) or endogenous opioids.

The role of the airway innervation and neurogenic inflammatory events is well established for experimental models of airway inflammatory and obstructive diseases such as bronchial asthma or chronic obstructive pulmonary disease (COPD). In this respect, a large number of mediators of inflammation are known to influence sensory and cholinergic nerve activity under conditions of airway inflammation. It is also accepted that the airway innervation and especially sensory nerve fibers regulates all major features of human respiratory functions. However, findings from animal experiments on the significance of neurogenic inflammation have not been fully extrapolated to the human situation so far. As the different pharmacological properties of airway nerve mediators, which orchestrate many aspects of airway inflammation, may also be used as targets for future therapeutic strategies, aspects of neurogenic inflammation display an important area of research currently.

The present review aims at addressing a comprehensive summary of the basic mechanisms of airway neurogenic inflammation and comparing data from animal models to those obtained in humans in order to provide insights into the current concepts of research in this area.

Airway innervation

Next to the classical sympathetic and parasympathetic innervation (Table 1), also sensory nerve fibers project to the airways and innervate all major respiratory effector

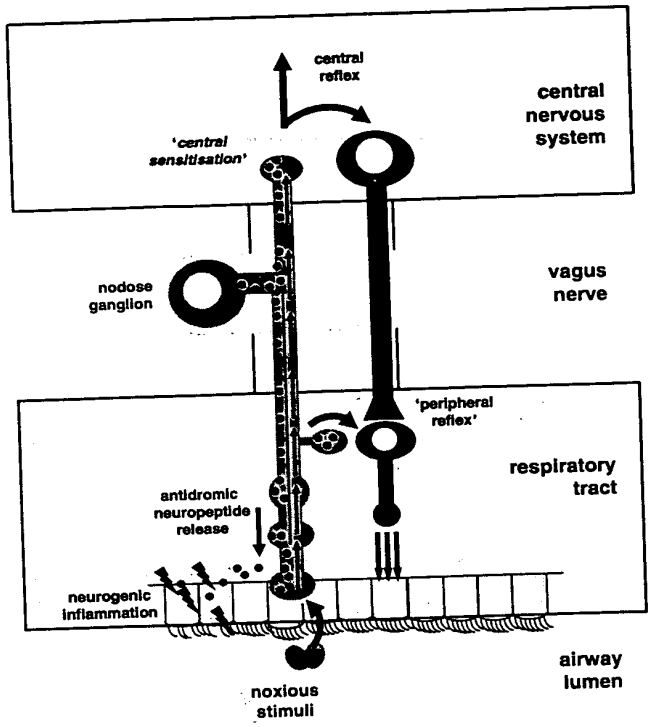

Figure 1. Current concept of neurogenic inflammation. After peripheral noxious stimulation by airborne substances such as allergens or tobacco smoke orthodromic activation of sensory nerve fiber endings takes place. The classical pathway then leads via orthodromic transmission into the brainstem. Here, the signal is modulated via interneurons and then transmitted via efferent parasympathetic nerves fibers to induce parasympathetic effects after synaptic transmission in local intrinsic airway ganglia. The neural events orchestrating neurogenic inflammation start in the sensory ganglia perikarya with the induction of pro-inflammatory neuropeptide gene expression. Then the neuropeptides are transported antidromically via the sensory nerve fibres back to the peripheral endings in the airways. Here, they are locally released and propagate the events of neurogenic inflammation.

cells (Fig. 2). The so-called sensory innervation of the mammalian airways, which has also been addressed as noncholinergic nonadrenergic (NANC) innervation, constitutes a very heterogeneous population of nerves. In this respect, sensory nerve fibers are variable in their myelination, caliber and central nervous input (9). The largest portion of mammalian airway-innervating sensory nerve fibers originates from vagal ganglia (10, 11). However, also a smaller number of airway sensory nerves originate from dorsal root ganglia (12–14) (Fig. 2).

In the brainstem, the majority of neurons that innervate the airways end in the commissural, ventrolateral and medial areas of the nucleus tractus solitarius (15–17). In these areas, they have synapses with interneurons communicating with medullary networks (18–20). Still, only little is known about the resulting multineuronal circle. It is generally assumed that the circle represents a reflex loop which is activated by the peripheral stimulation of sensory airway neurons leading to the enhancement or inhibition of cholinergic nerve fibers which project to numerous target cells in the respiratory tract (Fig. 3).

Apart from the central multineuronal reflex circuit there is also a second mode of action found in airway sensory neurons: peripheral stimuli in the respiratory tract can also lead to a local neuronal mechanism orchestrated by sensory neurons (21, 22) expressing pro-inflammatory neuropeptides such as CGRP or substance P. As nerve fiber terminals and also the receptors for these neuropeptides are localized in the vessel walls, bronchial smooth muscles, the epithelial area and around mucus glands (23), the local stimulation of sensory neurons projecting to these targets and the following neuropeptide release can lead to features of inflammation such as hyperemia (24), edema (25), mucus hypersecretion (26) and contraction of the bronchial smooth muscle. The underlying processes can even be initiated without a prior neuronal depolarization. Sensory nerve fibers are also known to project to local intrinsic airway ganglia (23, 27), which may depolarize in response to tachykinins (28, 29). Therefore, peripheral activation of sensory nerve fibers with consecutive neuropeptide release may also lead to a significant modulation of centrally mediated medullary reflexes (30).

Neuropeptides involved in neurogenic inflammation

Among the large variety of neuropeptides stored in and secreted from airway nerves, those which are expressed in

Table 1. Neuromediators

Mediator	Receptor	Major origin	Major effects
Acetylcholine	Nicotinergic and cholinergic receptors	Parasympathetic fibers	Bronchoconstriction
Catecholamines	Adrenergic receptors	Sympathetic fibers	Bronchodilation
Tachykinins	Tachykinin receptors (NK1, NK2)	Sensory fibers	Inflammation, bronchoconstriction, mucus secretion, plasma exudation
CGRP	CGRP-receptors	Sensory fibers	Vasodilation, bronchial tone
NPY	NPY-receptors	Sympathetic fibers	Inflammation
VIP	VPAC1, VPAC2	Parasympathetic, sympathetic & sensory fibers	Bronchodilation, vasodilation, immunomodulation
PACAP	VPAC1, VPAC2, PAC1	Parasympathetic, sympathetic & sensory fibers	Bronchodilation, vasodilation, immunomodulation

CGRP, calcitonin gene-related peptide; NPY, neuropeptide tyrosine; VIP, vasoactive intestinal polypeptide; PACAP, pituitary adenylate cyclase-activating peptide.

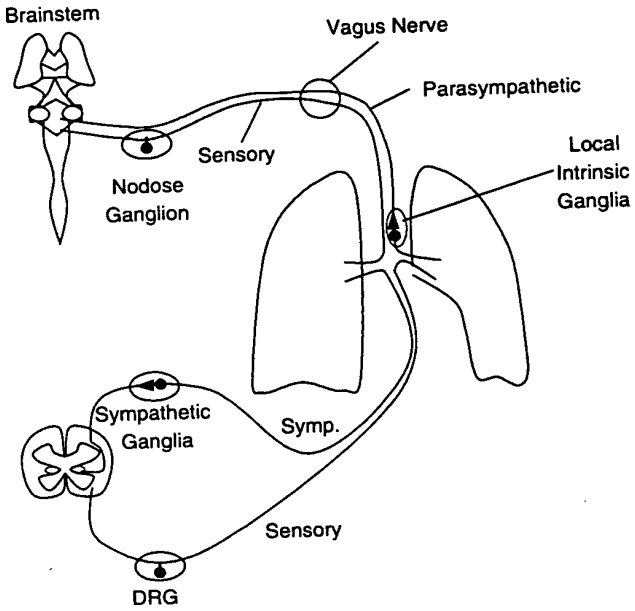

Figure 2. Anatomical basis of airway innervation. Schematic illustration of human airway innervation. The vagus nerve supplies all parasympathetic, preganglionic neurons and the majority of sensory nerve fibers. Sympathetic nerve fibers originate from sympathetic cervical and thoracic ganglia. A minority of sensory nerve fibers originates from dorsal root ganglia (DRG).

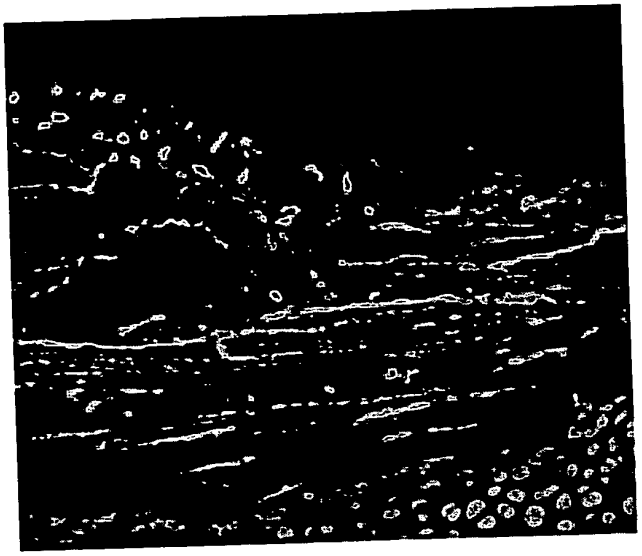

Figure 3. Airway innervation. Double-fluorescence immunohistochemistry illustrating the abundant presence of nerve fibers and their target cells in the airways. Nerve fibers are identified with the pan-neuronal marker protein gene product 9.5 (FITC-labeled) and immune cells with the common all leukocyte antigen (CD45, CY3-labeled) marker in the airways of tissues from a guinea pig model of chronic airway inflammation (ovalbumin-induced).

sensory nerves especially contribute to the events of neurogenic inflammation. Their *in vitro* and *in vivo* pro-inflammatory and immune effects have been characterized on numerous respiratory effector cells. They are secreted during airway inflammation in reaction to a multitude of inflammatory mediators. More than 50 different non-neuronal mediators of airway inflammation have been described so far (31), which may propagate the neuropeptide-release from airway sensory nerve fibers in diseases such as asthma or COPD (32). These mediators may not only induce neuropeptide release but also increase the expression of neuropeptide receptors on either neuronal, inflammatory or respiratory target cells. They may also influence the degradation of neuropeptides in the periphery. Although neuropeptides usually originate from airway nerves, recent studies indicate non-neuronal sources such as inflammatory or epithelial cells, especially in states of airway inflammation found in asthma and COPD (33).

Pro-inflammatory neuropeptides

Tachykinins

The major neuropeptides of the tachykinin family released into the airways are substance P and neurokinin A. Their release from sensory nerves is affected by numerous other mediators including opioids, dopamine or histamine (Fig. 4). Tachykinins influence many respiratory functions in the lung under both normal and pathological conditions (34). Their inducible expression has been demonstrated in inflammation, where they act as potent regulators of neurogenic inflammation because of their pro-inflammatory effects on many airway effector cells (11).

Both substance P and neurokinin A derive from the preprotachykinin (PPT) A gene. They share a common carboxyterminus amino acid sequence containing the biologically active domain (35). In the upper and lower respiratory tract, tachykinin immunoreactivity is present in nerve fibers localized to submucosal glands, airway smooth muscle and vasculature (23, 36). Retrograde neuronal tracing studies in rats and guinea pigs indicate that sensory nerve fibers which innervate the trachea mainly originate from the jugular and nodose vagal sensory ganglia (10, 14). Tachykinin-expressing nerve fibers mainly originate from neurons localized in the jugular and dorsal root ganglia (10, 13).

It was shown recently that a furin-mediated cleavage of the bovine respiratory syncytial virus fusion protein leads to the release of a peptide converted into a biologically active tachykinin termed virokinin (37). The cellular enzymes involved in the C-terminus maturation of virokinin are present in many established cell lines, and

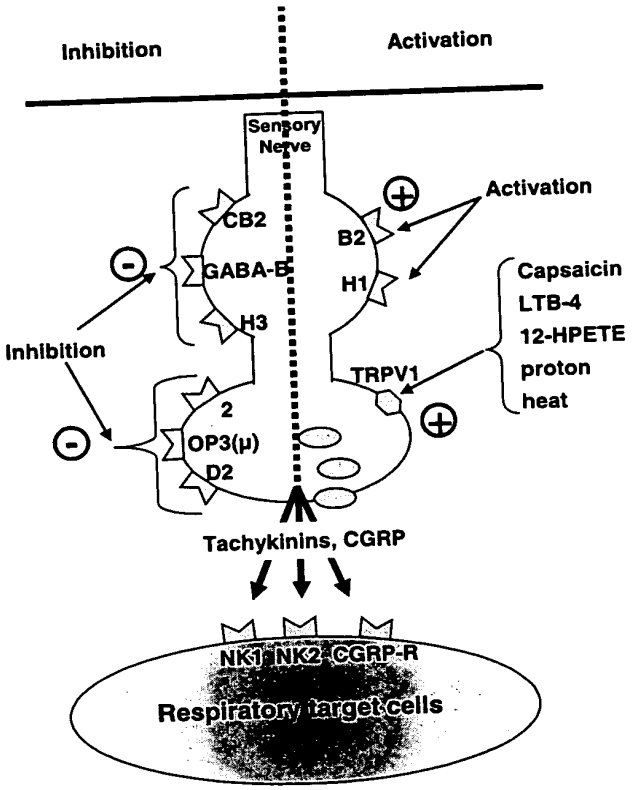

Figure 4. Modulation of sensory nerve activity. Sensory nerve-mediated airway effects are induced via antidromic release of pro-inflammatory neuropeptides such as tachykinins or calcitonin gene-related peptide (CGRP) and their receptors NK-1, NK-2 and CGRP-R. Sensory nerve activity is regulated via stimulation of the vanilloid TRPV1, bradykinine (B2), or histamine (H1) receptor, and inhibited via activation of numerous receptors including adrenergic (α2), opioid (OP3), cannabinoid (CB2), dopaminergic (D2), or histamine (H3) receptors.

indeed, virokinin is secreted by virus-infected cells. *In vitro* experiments revealed that virokinin by itself is capable of inducing smooth muscle contraction (37). Therefore, the viral tachykinin-like peptide virokinin may be considered a novel form of molecular mimicry, by which a virus may benefit affecting host immune responses (37, 38).

Tachykinins are known to bind to three different G-protein-coupled receptors. These receptors can be distinguished by their molecular properties and different pharmacological affinities for the tachykinin ligands (39). While substance P is principally activating the NK1 receptors, neurokinin A mainly acts via NK2 and neurokinin B via NK3 receptors (40–42). A differential pattern of tachykinin receptor distribution is present in the respiratory tract: While NK1 receptors are predominantly localized to the airway epithelium, submucosal glands, and vessels, NK2 receptors are mainly expressed on the airway smooth muscle (43–45).

The majority of data in humans are available on the regulation and function of tachykinin ligands and their receptors in allergic inflammatory diseases (46–49). The interactions of tachykinins and their receptors in diseases such as COPD are largely unknown. However, it has been reported that tachykinin gene expression is increased after chronic exposure to cigarette smoke in animals (50) and this mechanism may also exist in humans.

Pulmonary effects of tachykinins

Tachykinins such as substance P and neurokinin A exert numerous effects on respiratory target cells. Tachykinins, particularly NKA, potently constrict human airway smooth muscle *in vitro* via NK_2-receptors, with significantly greater potency in smaller airways (51). This suggests that sensory nerve-derived tachykinins may have a role in regulating constrictory effects in more peripheral airways, whereas cholinergic fiber effects dominate in larger airways. Interestingly, the smaller airways play a prominent role in COPD.

In asthma, neurokinin A leads to bronchoconstriction after inhaled or intravenous administration (52). *In vitro*, after removal of the epithelial layer, the bronchoconstrictory responses to tachykinins are significantly increased (53). This effect points to an increased importance of tachykinins in diseases with damaged airway epithelial layers such as bronchial asthma or COPD.

Tachykinins are also known to potently stimulate secretion from human submucosal glands *in vitro*. In this respect, they stimulate epithelial goblet cell secretion. This effect is accomplished via NK_1-receptors. The exact pathways leading to the induction of mucin genes such as the primary gel-forming mucins expressed in the upper and lower respiratory tract, MUC5AC or MUC5B (54–57), have not been elucidated so far. Further respiratory effects depending on NK_1-receptor signaling are plasma vasodilatation and exudation, as well as acetylcholine release facilitation at cholinergic nerve terminals, thereby enhancing cholinergic neurotransmission (58).

Metabolism of tachykinins

The predominant mode of tachykinin cleavage in the airways is propagated by at least two enzymes which are neutral endopeptidase (NEP) and angiotensin converting enzyme (ACE) (59). In general, inflammatory stimuli might simultaneously increase the synthesis of tachykinins and decrease the activity of (NEP) leading to an increase in the overall pulmonary effects of tachykinins. As NEP is expressed in the airway mucosa and submucosa, it plays the major role in the degradation of tachykinins in the airways. By contrast, ACE is predominantly found in vascular cells and therefore regulates the cleavage of intravascular peptides. The activity of NEP

can affect the responsiveness to tachykinins in the airways. In this respect, NEP inhibition has been demonstrated to significantly increase tachykinins-induced bronchoconstriction both *in vitro* and *in vivo*. Removing the epithelium decreases the activity of NEP. Also, tobacco smoke or exposure to viral infections can decrease NEP activity and thereby diminish the cleavage and degradation of pro-inflammatory tachykinins (59).

Calcitonin gene-related peptide

Calcitonin gene-related peptide is another major neuropeptide that belongs to the class of pro-inflammatory sensory neuropeptides (60).

CGRP consists of 37 amino acids and is a product of calcitonin pre-mRNA alternative splicing. The neuropeptide is expressed and co-localized together with tachykinins in sensory nerve fibers, which innervate the upper and lower airways of several species, including humans (61–63). Next to its expression in airway sensory nerve fibers, CGRP has also been detected both at the transcriptional and translational level in pulmonary neuroendocrine cells. It is expressed in both the solitary type and cluster type (neuroepithelial body – NEB) at all anatomical levels of the respiratory tract reaching from the larger to the lower airways and even alveoli (64, 65). The molecular identity of CGRP receptors has not been elucidated until recently. Before, numerous receptors have been suggested to be associated with CGRP or related peptides but had to be re-classified as orphan receptors (66–68). It was recently shown that CGRP receptors belong to the family of G-protein coupled seven transmembrane receptors as the receptors for many other neuropeptide receptors do (69–71). Interestingly, the activity of CGRP receptors is regulated by a family of receptor activity-modifying proteins (RAMP) leading to different type I or type II CGRP receptors (72).

Binding sites for CGRP in human airways have been identified using autoradiography (73): they are widely distributed through the respiratory tract with a dense labeling in the area of bronchial and pulmonary blood vessels of all sizes and in alveolar walls. In contrast to these densely labeled areas, the airway smooth muscle and epithelial layer was only sparsely labeled. No labeling was found in regions of submucosal glands, corresponding to the CGRP-immunoreactive nerve fiber distribution (73). Immunohistochemistry to identify CGRP type I receptor expression in human bronchial blood vessels revealed receptor-immunoreactivity in the endothelium of venules but not in the endothelium of arterioles (60).

Pulmonary effects of CGRP

Numerous studies have provided direct evidence for an important regulatory role of CGRP in multiple respiratory functions over the past years. These include the regulation of the airway smooth muscle tone or of the vessel tone (60). However, the exact mode of CGRP effects on airway smooth muscle tone is still unclear as controversial bronchoconstrictory or -dilatory effects have been reported in the past years in different species and preparations (60). Recently it was shown for human bronchi *in vitro* that CGRP causes a concentration-dependent contraction of epithelium-denuded human bronchi whereas no significant effects are found in epithelium-intact bronchi (74), indicating a potential altered effect of CGRP on airway tone in respiratory diseases with a damaged epithelial layer such as asthma or COPD.

In human pulmonary arteries, CGRP causes a concentration-dependent relaxation of both endothelium-intact and -denuded arteries. Pretreatment with indomethacin prevents the CGRP-induced relaxation in pulmonary arteries suggesting that prostaglandins are involved in the intracellular signal transduction pathway. By contrast, nitric oxide (NO) is not involved in this mechanism as pretreatment with the NO synthase inhibitor L-NAME does not affect CGRP-induced vascular relaxation. The CGRP-induced effects on both bronchi and vessels are prevented by application of the CGRP-antagonist CGRP (74).

There are no reports currently available about the effects of CGRP on human airway mucus secretion. However, although glandular areas only display a very low density of CGRP binding sites (73), CGRP might affect mucus secretion indirectly by increasing the blood flow to submucosal glands.

Altogether, the complex effects of CGRP on the airway and vascular tone and the immune system has nowadays prevented the development of any therapeutic strategy based on CGRP targets (60).

Metabolism of CGRP

Calcitonin gene-related peptide can be subjected to inactivation by several enzymes which are expressed in the human respiratory tract. However, the exact cleavage pathways have not been revealed so far. Neutral endopeptidase (EC 3.4.24.11) inhibitors have been shown to increase some of the CGRP effects in the airways (75). Rat alpha-CGRP contains the tetrapeptide eosinophil granule chemotactic factor Val32-Gly-Ser-Glu35 sequence. This fragment is generated following cleavage at a substrate recognition site which is not common for NEP. In addition, the chemotactic activity of rat alpha-CGRP is increased after proteolysis. By contrast, rat beta-CGRP that lacks the tetrapeptide eosinophil granulocyte chemotactic factor Val32-Gly-Ser-Glu35 is devoid of chemotactic activity. The tetrapeptide has also been identified as the primary fragment with chemotactic activity towards eosinophil polymorphonuclear leukocytes (76).

Anti-inflammatory neuropeptides

Next to pro-inflammatory sensory neuropeptides such as tachykinins and CGRP, airway-projecting sensory neurons also contain a variety of other neuropeptides which may modulate the neurogenic inflammation. Apart from peptides such as secretoneurin which is derived from secretogranin II (chromogranin C), localized to sensory nerves and a potent attractant of eosinophils (77), anti-inflammatory nonsensory peptides may participate in events underlying neurogenic inflammation.

One of the primary anti-inflammatory peptides is VIP (78). It is one of the most abundant neuropeptides found in the upper and lower human respiratory tract (79–81) and a likely neurotransmitter or neuromodulator of the inhibitory nonadrenergic noncholinergic airway nervous system. Together with pituitary adenylate cyclase-activating peptide (PACAP), VIP influences many aspects of pulmonary biology (82), and its immunoreactivity is present in human airway nerve fibers innervating the pulmonary and bronchial vessels, the tracheobronchial airway smooth muscle layer, and submucosal glands (83, 84). Also, receptors for VIP have been identified in airway epithelial cells and in inflammatory cells (69–71, 85). Next to its prominent bronchodilatory effects, VIP potently relaxes pulmonary vessels.

Similar to pro-inflammatory peptides, VIP is subjected to degradation by airway enzymes such as NEP (86), mast cell chymase and mast cell tryptase (87). The NEP hydrolysis fragments of VIP are physiologically inactive (88). Also, chymase and tryptase VIP-degradation products fail to relax vascular or airway smooth muscle (89).

The precise role of VIP in the pathogenesis of asthma and COPD is still uncertain. Although a therapy using the strong bronchodilatory effects of VIP would offer potential benefits, the rapid inactivation of the peptide by airway peptidases has prevented a widespread use of effective VIP-based drugs so far. In this context, the VIP-related peptide PACAP-38 may be an interesting candidate for further studies, as it has a prolonged half-life in the airways, when compared with VIP.

Interacting mediators

A further family of biologically active peptides which may interact with sensory neurons and thereby propagating airway neurogenic inflammation are the neurotrophins (90). They encompass structurally and functionally related polypeptides with an up to 50% homology of amino acid sequence. Neurotrophins induce neuronal outgrowth (or development) and differentiation, regulate apoptotic mechanisms and influence synaptic function of the central and peripheral nervous system (91). The most prominent members of the neurotrophin family are nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin (NT)-3, and NT-4/5.

In the respiratory tract, the neurotrophins are localized to a variety of cell types including mononuclear cells and epithelial cells (Table 2).

The biological activities of these four neurotrophins are mediated by tyrosine-specific protein kinase (Trk) receptors encoded by the *trk* gene and by the low affinity $p75^{NTR}$ neurotrophin receptor (92) (Fig. 5). NGF binds to a high affinity termed TrkA receptor, dimerizing and activating the intrinsic tyrosine kinase by auto-phosphorylation. NT-4 and BDNF bind to and activate the TrkB receptor. The TrkC receptor is specific for the mediation of NT-3 effects, while all NTs can act at the low affinity $p75^{NTR}$ neurotrophin receptor (92). In several chronic inflammatory diseases including chronic airway inflammation (93), an important role for neurotrophins as inflammatory mediators has been recently demonstrated. Asthmatic patients exhibit significantly enhanced NGF levels in the bronchoalveolar lavage fluid and also, intense NGF-immunoreactivity is observed in bronchial epithelium, smooth muscle cells and infiltrating

Table 2. Nerve growth factor sources and targets

Target cell	Source	Effect on
B cells	+	+
Th1 cells	+/−	−
Th2 cells	+	−
Mast cells	+	+
Eosinophils	+	+
Basophils	−	+
Neutrophils	−	+
Macrophages	+	+
Neurons	−	+
Fibroblasts	+	+
Epithelial cells	+	−
Smooth muscle cells	+	−

+, Production/effects reported; −, production/effects not reported/not investigated.

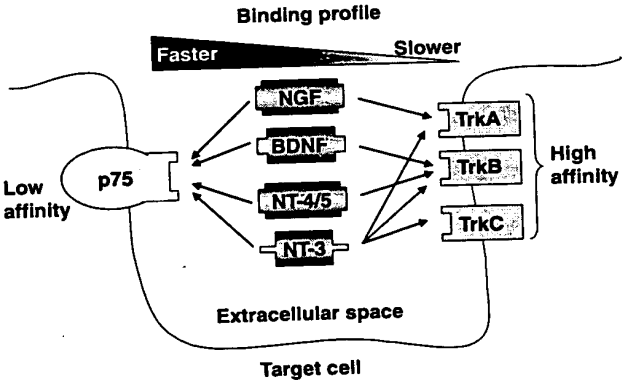

Figure 5. Neurotrophins and their receptors. Neurotrophins bind with different affinities to tyrosine-specific protein kinase (Trk) receptors encoded by the *trk* gene (high affinity) and the $p75^{NTR}$ neurotrophin receptor (low affinity).

inflammatory cells in the submucosa (94). Nerve growth factor is also expressed in human airway smooth muscle cells (95), human lung epithelial A549 cells (96) and in human fibroblasts (97) under inflammatory conditions. By contrast, the precise translational and transcriptional expression levels have not been assessed in COPD so far and future studies need to address this issue.

Neurogenic inflammation in experimental models

To identify the molecular mechanisms underlying the different events of neurogenic inflammation in the mammalian airways, animal models have been studied in great detail. These experiments pointed to an important role of airway neurogenic inflammatory events which are orchestrated by antidromic released pro-inflammatory neuropeptides such as tachykinins and CGRP (Fig. 1). Also, exogenous substance P administered by aeosol to guinea-pigs induces by itself a bronchial hyperresponsiveness and airway inflammation (98). Based on data obtained from these studies, the existence of a neurogenic inflammatory component in human airway diseases such as asthma and COPD seems very likely. Five major approaches exist to investigate the precise role of the autonomous airway innervation in models of airway inflammation. These encompass strategies using loss-of-function and gain-of-function gene-modulating techniques, neuropeptide receptor antagonists, inhibition of neuromediator release, capsaicin depletion strategies, and peptidase inhibitor strategies.

Gain-of-function and loss-of-function models

The targeted deletion of tachykinin receptor or ligand genes within a loss-of-function model displays an important current approach to dissect the distinct biological effects of these neurotransmitters *in vivo*. An important hallmark was the targeted deletion of the gene encoding the NK_1 receptor. It was revealed that these mice were healthy and fertile, but the characteristic amplification and intensity coding of nociceptive reflexes was absent (99). Although substance P was shown not to mediate the signaling of hyperalgesia or acute pain, it was essential for the full development of stress-induced analgesia and for an aggressive response to territorial challenge. This pointed to an unexpected role in the adaptive response to stress of the peptide. The deletion of the NK_1 receptor gene in mice was also shown to affect respiratory responses to hypoxia but not to alter the respiratory network maturation (100). The role of the tachykinin NK_1 receptor was also investigated in a model of antigen-induced airway inflammation in these mice (101). While the loss-of-function of the NK_1-receptor enhanced baseline carbachol-induced airway responsiveness, it did not modulate the effect of allergen in the airways: airway inflammation as inflammatory cells in bronchoalveolar lavage fluid, and antigen-specific serum IgE were unchanged. In future, also intracellular signal transduction pathways need to be studied in greater detail. Candidate genes involved in both asthma and COPD are i.e. MAPK signaling pathways such as p38 or c-Jun N terminal kinase (JNK) or SMAD proteins. They are likely to be important signal transducers in airway effector cells and airway-innervating neurons (102–104).

A second important peptide family that has been studied in great detail for its role in the orchestration of chronic inflammatory airway changes are the neurotrophins. By use of different approaches of neurotrophin receptor or ligand overexpression or targeted gene-depletion, an important role of this regulatory system was proposed for the regulation of airway inflammation (105, 106).

Neuropeptide receptor antagonism

Antagonism of the selective neuropeptide receptors represents a useful tool to discriminate the precise role of neuropeptides. This approach was used to identify tachykinin effects: the NK_1-receptor antagonist CP 96345 significantly blocked plasma exudation in response to vagal stimulation and to cigarette smoke (107). This receptor-specific antagonization did not have any impact on bronchoconstrictory responses, but was blocked by selective NK_2-antagonists (108). The antagonization of NK_1-receptors also led to a decrease in bradykinin- and hyperpnoea-induced plasma exudation, but did not alter acute allergen-induced plasma exudation in sensitized guinea pigs (109).

Blockage of neuropeptide release

A further approach to assess the impact of neurogenic inflammatory events on airway inflammation is blockade of neuropeptide release. In this respect, a number of drugs has been identified to act directly on receptors located on prejunctional airway sensory nerves and inhibit neuropeptide release (19). Among these substances, opioids have been attributed to be the most effective class of inhibitors. They exert their influence predominantly acting via prejunctional OP_3 receptors that were formerly termed μ-receptors (Fig. 4). With regard to mucus secretion representing a major feature of both asthma and COPD, opioids have been reported to inhibit cigarette smoke-, capsaicin- and electrically-induced goblet cell secretion in the guinea pig trachea. It was suggested that they inhibit cholinergic-mediated goblet cell secretion via prejunctional OP_3 (μ) and OP_1 (δ) receptors and sensory nerve-mediated goblet cell secretion via OP_3 receptors (110). In human tissues, the OP_3 (μ) opioid ligands reduce cholinergic responses to electric field stimulation (EFS), most likely via inhibiting the release of acetylcholine from postganglionic parasympathetic nerve fibers innervating the airway smooth muscle

(111). Apart from the respiratory expression and function of classical opioids and opioid receptors, further groups of opioid-like peptides such as endomorphins and nociceptin have been identified in the respiratory tract (112). In contrast to endomorphin-1 that acts via the classical OP_3 (μ) receptor, nociceptin has been reported to bind to a new receptor termed opioid receptor-like-receptor (ORL1). Nociceptin inhibits EFS-stimulated cholinergic parasympathetic bronchoconstriction in the guinea pig (113). This inhibition was not affected by the non selective opioid receptor antagonist naloxone and nociceptin was thus suggested to act prejunctionally via a novel opioid receptor, possibly ORL_1 (113).

Also, studies demonstrated that nociceptin inhibits nonadrenergic noncholinergic contraction in guinea-pig airways (114). This inhibitory effect was mediated through a prejunctional mechanism involving none of the classical (OP_1-OP_3) opioid receptors. Opioid receptor-like-receptor-mRNA was found to be expressed in the upper vagal sensory ganglia, where the cell bodies of the tachykinin-containing sensory neurons are located. Also, nociceptin-immunoreactive nerve fibers in the airway wall, distinct from the tachykinin-containing fibers, were identified as a pulmonary source of nociceptin (115). By use of the specific nociceptin receptor antagonist [Phe1p-si(CH2-NH)Gly2]NC(1–13)NH2 ([F/G]NC(1–13)NH2), the nociceptin-induced inhibition of tachykinergic contraction was subsequently identified (116). A further report using the new ORL1 receptor nonpeptide antagonist J-113397 indicates that nociceptin may inhibit capsaicin-evoked tachykinin release from sensory nerve terminals in guinea pig lung by a prejunctional mechanism (117).

Also, endomorphin-1 and -2 (EM-1, EM-2) were identified as potent inhibitors of EFS-induced tachykinergic contractions of guinea pig isolated bronchi. Whereas EM-1 was reported to act as OP_3 (μ) receptor agonist, EM-2 was not blocked by any antagonist of the classical opioid receptors in isolated bronchi (112). For human and guinea-pig trachea, endomorphin-1 and -2 have also been shown to inhibit cholinergic contractile responses. This inhibition was prevented by naloxone suggesting that both peptides act via the OP_3 (μ) opioid receptor in the cholinergic component of tracheal smooth muscle constriction (118). However, in striking contrast to the promising modulatory effects of classical and new opioids, effective therapeutic strategies have not been developed so far. Next to opioids, a number of other molecules may also actively inhibit nerve activity, i.e. via targeting calcium-activated large conductance potassium channels expressed in sensory airway nerve fibers (119). To the same extent, targeting other ion channels expressed on sensory neurons may lead to an inhibition of neurogenic inflammatory events in the airways (110). Also, local anesthetics may potentially inhibit sensory neuropeptide release, suggesting that major similarities in the events contribute to airway inflammation observed in asthma and COPD, and hyperalgesia observed in clinical pain syndromes.

Neuropeptide depletion studies

In rodents, pretreatment with capsaicin leads to a depletion of neuropeptides from sensory nerves of adult animals. This approach can be performed in neonatal animals and leads to a nerve fiber degeneration. In rat or guinea pig, pretreatment with capsaicin leads to an inhibition of tobacco-smoke microvascular leakage and goblet cell secretion (9). Capsaicin-depleted sensory nerve fibers may also contribute to microvascular leakage induced by hypertonic saline, hyperventilation, or hypocapnia (120). In addition, capsaicin affects airway responsiveness to cholinergic agonists in guinea pigs (120). Pretreatment with capsaicin also blocks airway hyperresponsiveness induced by viruses in guinea pigs (121). This points to a role of capsaicin-sensitive sensory airway nerves in the regulation of airway responsiveness.

Targeting neuropeptide cleavage

Targeting the cleavage of neuropeptides represents a further tool to characterize the role of neurogenic inflammation in the airways as the activity of neuropeptide-degrading enzymes may determine the extent of airway neurogenic inflammation. Inhibiting NEP by its inhibitors thiorphan or phosphoramidon leads to an increase in neurogenic inflammation (59). Enzymes such as NEP are not specific for tachykinins and may also cleave many other biologically active peptides. Therefore, the inhibition of neuropeptide cleavage does not distinguish between pro- and anti-inflammatory classes of neuropeptides and interacting peptides *in vivo*. After inactivation by proteolysis, the fragments are removed by peptide transporters which are expressed in the respiratory tract (122, 123) and other organs (124, 125). In contrast, viral infections affect neural responses in guinea pigs by inhibiting peptide cleavage. Sendai virus infection for instance increases airway neurogenic inflammation, and mycoplasma infections lead to enhanced microvascular leakage that is mediated by NEP in rats (126, 127). A further virus related to neurogenic inflammation is the influenza virus as its neuraminidase enhances tachykinin-induced bronchoconstriction (128). In summary, the different effects on airway neurogenic inflammation caused by respiratory infections as observed in animal models may be explanatory for the potentially deleterious effects of respiratory infections in patients with COPD and asthma.

Neurogenic inflammatory events in human airways

While a large amount of indicative data on the important role of neurogenic inflammatory events in the

development and progression of chronic airway inflammation has been reported in the past decades in animal models of airway inflammation, much less is known about the role of these neural mechanisms in human airway diseases.

Human respiratory sensory innervation

There seems to be major species-specific differences between human and animal respiratory tract innervation, as many of the findings on airway innervation or promising effects of compounds targeting sensory nerves were not replicated with human tissues. In specific, studies have failed to confirm an increase in substance P in lung tissues from patients with asthma (129) or an increase in substance P-immunoreactive nerve fibers in airways of asthmatic patients (130). Also, neither contractile responses to capsaicin nor distinct e-NANC responses have been demonstrated in human airways *in vitro*.

While the guinea pig innervation is the closest approach to the human situation among all common laboratory species, there are still differences present: In general, sensory nerve fibers containing substance P and CGRP are of lower number in the human respiratory tract as compared with guinea-pig airways. Whereas these fibers constitute a large portion of total epithelial nerve fibers in the guinea pig airways, they have been estimated at only about 1% in humans (131). However, this preliminary data needs further morphological studies to clarify this issue. In particular, the extrinsic and intrinsic murine airway innervation is surprisingly unknown, although this species is commonly used to assess mechanisms of airway inflammation and interactions between nerve fibers and the immune system.

In contrast to these potential anatomical and functional discrepancies, airway neuronal plasticity is generally accepted to occur in human pulmonary diseases. To assess if alterations in neuronal subpopulations are disease-specific or an epiphenomenon of the inflammation, different subtypes of chronic upper airway diseases including hyperreflectoric rhinitis (36), aspirin-sensitive rhinitis (80) and toxic rhinitis (62) have been examined. Hyperreflectoric rhinitis is a chronic upper airway inflammatory disease related to a nonspecific hyperreactivity. Expression profiling for substance P, CGRP, VIP, and neuropeptide tyrosine (NPY) revealed an abundant presence of nerve fibers expressing these peptides in the airways. Neuropeptide-expression in mucosal nerves was also quantitatively assessed and significant increases were found for substance P (3.00 ± 0.37 *vs* 1.64 ± 0.34 staining intensity in the control group) and VIP (2.33 ± 0.42 *vs* 0.82 ± 0.33) (36). These results demonstrate differences in levels of neuropeptides in the innervation of human nasal mucosa between nonrhinitic and hyperreflectoric rhinitic subjects and pointed to a modulatory role of neuropeptide-specific subpopulations of nerve fibers in hyperreflectoric rhinitis. Irritative toxic rhinitis is induced by chemical compounds such as tobacco smoke, ozone, solvents, formaldehyde, nickel, or chrome which are also known to be associated to the development and progression of COPD and asthma. Semiquantitative immunohistochemistry for substance P, CGRP, VIP and NPY demonstrated significant differences between rhinitis patients and controls: Toxic rhinitis patients had significantly increased expression scores for VIP (2.83 ± 0.31 *vs* 1.27 ± 0.47 control group) and NPY (3.17 ± 0.31 *vs* 0.91 ± 0.37 control group) (62). These results indicated a differential participation of subclasses of mucosal nerves in the pathophysiology of toxic rhinitis and suggested that the changes in nerve profiles found in toxic rhinitis and hyperreflectoric rhinitis are disease-specific and not an epiphenomenon of inflammation.

Next to toxic rhinitis and hyperreflectoric rhinitis, aspirin-sensitive rhinitis was investigated which represents the manifestation of aspirin intolerance in the upper airways (80). The disease is a pseudoallergy against aspirin and related nonsteroidal anti-inflammatory drugs. Immunohistochemical analysis demonstrated that aspirin-sensitive rhinitis patients also had a significant increase in VIP-immunoreactive nerve fibers (80).

These changes observed for the different forms of chronic upper airway inflammation may be partly regulated via neurotrophins. NGF significantly increases the transcription of the preprotachykinin-A (PPT-A) gene which encodes for substance P and neurokinin A *in vitro* (132). The NGF expression is increased in asthma (133), and might therefore account for the induction of substance P in airway nerves under inflammatory conditions. In this respect, earlier studies reported an increase in the number of substance P-immunoreactive nerve fibers in the airways of patients with fatal asthma (134). Also, increased concentrations of substance P were documented in the bronchoalveolar lavage fluids of patients with asthma, with a further rise following allergen challenges (135). Substance P has also been found in the sputum of asthmatic patients after inhalation of hypertonic saline (136). However, it was difficult to replicate these findings in other populations and other studies did not reveal any increases (129, 130). At the receptor level, it was reported that the NK_1-receptor gene expression is increased in the airways of asthmatic patients (137). Also, an abnormal expression of NK_2-receptors was documented in asthma (138).

Potential pharmacological options

Although the precise role of neurogenic inflammatory events has not been fully defined in human asthma and COPD so far, tachykinin receptor antagonism, sensory nerve activity-modification or neuropeptide release-inhibition still represent potential targets for

pharmacological interventions. Also, novel molecular targets for the delivery of drugs have been identified (139) that offer promising pathways to deliver compounds directly to the airways via proton-coupled uptake mechanisms (140).

Tachykinin receptor antagonists display promising tools but a number of clinical studies conducted in asthmatic patients did not lead to any promising result in the first place. The first study used the nonselective tachykinin antagonist FK-224 and demonstrated inhibitory effects on bradykinin-induced bronchoconstriction in asthmatic patients (141). Also a more selective NK_1-receptor antagonist revealed a reduction in the duration of exercise-induced asthma (142). However, there was no consistent effect on the maximal bronchoconstriction. Additionally, a further potent NK_1-receptor antagonist (CP 99994) did not show any inhibitory effect on either hypertonic saline-induced bronchoconstriction or on cough (143). However, the administration of a dual tachykinin NK_1/NK_2-antagonist DNK333 was recently reported to inhibit neurokinin A-induced bronchoconstriction in asthma patients in a randomized, double-blind, placebo-controlled, crossover trial (144). This reported difference of four doubling doses of NKA at 1 h was decreased to a difference of 0.9 doubling doses at 10 h post-treatment (144). The results indicated that, although tachykinin NK_2-receptors mediate most of the direct smooth muscle contracting effect of neurokinin A, tachykinin NK_1-receptors are also involved in tachykinin-induced bronchoconstriction in humans.

Further approaches are the inhibition of sensory nerve activation. This may be accomplished by use of local anesthetics. But it has proved to be complicated to control the local deposition and to achieve adequate local respiratory concentrations (145). Surprisingly, even paradoxical bronchoconstrictions have been found in response to the administration of these compounds, which may be based on inhibition of tonic nonadrenergic bronchodilator reflexes.

Substances such as opioids that inhibit the release of neuropeptides via prejunctional mechanisms may also be implicated in the future treatment of chronic airway diseases. However, OR_3 (μ)-opioid agonists such as the pentapeptide BW443C did not prove to be effective in inhibiting metabisulphite-induced bronchoconstriction while they exhibited potent inhibitory effects on EFS (111). The compound may have been inactivated by epithelial NEP activity and future studies using novel synthetic substances are required to identify potential therapeutic options.

Conclusion

There are a large amount of data pointing to an important role of the sensory innervation and neurogenic inflammatory events in the development and progression of experimental airway inflammation in laboratory animals. In contrast, still only little is known about the precise role of neurogenic inflammation in human airway diseases such as asthma or COPD. This lack of in-depth knowledge depends on major difficulties to extrapolate the data obtained from experimental *in vivo* and *in vitro* studies to human asthma and COPD. However, there is no doubt that afferent nerve fibers participate in the orchestration of inflammatory changes occurring in human airway diseases, as many symptoms are known to be directly dependent on nerve fiber activity. Also, novel molecular targets for the delivery of peptide-based drugs have been identified (139) that offer promising pathways to deliver compounds to the airways (140). Therefore, identifying the precise role of neurogenic inflammation in asthma and COPD requires further attention and may focus in future on precise mechanisms of afferent nerve sensitization, the role of neuronal ion channels, and the interactions found between inflammatory cells and airway neurons.

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